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Insect trypanosomatids in Papua New Guinea: high endemism and diversity

Jana Králová [a,1], Anastasiia Grybchuk-Ieremenko [a,1], Jan Votýpka [b,c], Vojtěch Novotný [d,e,f], Petr Kment [g], Julius Lukeš [b,f], Vyacheslav Yurchenko [a,h,i,*], Alexei Yu. Kostygov [a,j,*]

[a] Life Science Research Centre, Faculty of Science, University of Ostrava, 710 00 Ostrava, Czechia
[b] Biology Centre, Institute of Parasitology, Czech Academy of Sciences, 370 05 České Budějovice, Czechia
[c] Department of Parasitology, Faculty of Science, Charles University, 128 44 Prague, Czechia
[d] Biology Centre, Institute of Entomology, Czech Academy of Sciences, 370 05 České Budějovice, Czechia
[e] New Guinea Binatang Research Center, Madang, Papua New Guinea
[f] University of South Bohemia, Faculty of Sciences, 370 05 České Budějovice, Czechia
[g] Department of Entomology, National Museum, 193 00 Prague, Czechia
[h] Institute of Environmental Technologies, Faculty of Science, University of Ostrava, 710 00 Ostrava, Czechia
[i] Martsinovsky Institute of Medical Parasitology, Tropical and Vector Borne Diseases, Sechenov University, Moscow, Russia
[j] Zoological Institute of the Russian Academy of Sciences, St. Petersburg 199034, Russia

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ABSTRACT

The extreme biological diversity of Oceanian archipelagos has long stimulated research in ecology and evolution. However, parasitic protists in this geographic area remained neglected and no molecular analyses have been carried out to understand the evolutionary patterns and relationships with their hosts. Papua New Guinea (PNG) is a biodiversity hotspot containing over 5% of the world's biodiversity in less than 0.5% of the total land area. In the current work, we examined insect heteropteran hosts collected in PNG for the presence of trypanosomatid parasites. The diversity of insect flagellates was analysed, to our knowledge for the first time, east of Wallace's Line, one of the most distinct biogeographic boundaries of the world. Out of 907 investigated specimens from 138 species and 23 families of the true bugs collected in eight localities, 135 (15%) were infected by at least one trypanosomatid species. High species diversity of captured hosts correlated with high diversity of detected trypanosomatids. Of 46 trypanosomatid Typing Units documented in PNG, only eight were known from other geographic locations, while 38 TUs (~83%) have not been previously encountered. The widespread trypanosomatid TUs were found in both widely distributed and endemic/sub-endemic insects. Approximately one-third of the endemic trypanosomatid TUs were found in widely distributed hosts, while the remaining species were confined to endemic and sub-endemic insects. The TUs from PNG form clades with conspicuous host-parasite coevolutionary patterns, as well as those with a remarkable lack of this trait. In addition, our analysis revealed new members of the subfamilies Leishmaniinae and Strigomonadinae, potentially representing new genera of trypanosomatids.

1. Introduction

In 1863, at the meeting of the Royal Geographical Society in London, Alfred Russel Wallace presented a map with a boundary between the Asian and Australian biological systems (Wallace, 1863). A few years later, Thomas Huxley validated Wallace's Line, clearly separating Asiatic species from their neighbours in a transitional zone later called Wallacea (Huxley, 1868). There is no general consensus concerning biogeographic regionalization. Here we use the scheme, in which the area to the east of Wallace's Line (Pacific Islands and New Guinea) belongs to Oceania (Holt et al., 2013). It is generally accepted that the western islands of Indonesia and the Malay Peninsula are dominated by Asian species compared with Australian fauna and flora prevailing in the neighbouring eastern islands. Numerous studies suggested a more complex scenario, revealing colonisation in both directions, as well as repeated transgressions of Wallace's Line (Bacon et al., 2013). Moreover, the

* Corresponding authors at: Life Science Research Centre, Faculty of Science, University of Ostrava, Chittussiho 10, 710 00 Ostrava, Czechia.
E-mail addresses: vyacheslav.yurchenko@osu.cz (V. Yurchenko), kostygov@gmail.com (A.Yu. Kostygov).
[1] These authors contributed equally to this work.

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distribution and diversity may be affected by island radiations that are thought to undergo fast evolution, diversification and rapid demise, before being superseded by different lineages of colonisers (Bellemain and Ricklefs, 2008).

Most studies of Wallace's Line focused on plants or animals, while protists remained neglected. Trypanosomatids represent a group of protists particularly suitable for biogeographic studies. While there were several broadscale surveys of trypanosomatid diversity in insects in various geographic areas (Lukeš et al., 2018), none were conducted in Oceanian and Australian regions. Meanwhile, Papua New Guinea (PNG) represents a biodiversity hotspot containing over 5% of the world's biodiversity in less than 0.5% of the total land area. It is particularly interesting from the perspective of the evolutionary dynamics of colonisation, biological radiations, and effect of the geographical barriers on the distribution and diversity of trypanosomatids. PNG hosts over 20,000 species of higher plants, 800 species of birds, and over 300,000 species of insects with a high proportion of endemics (Novotný et al., 2006, 2007; Marshall and Beehler, 2007).

The family Trypanosomatidae unites highly prevalent and widespread unicellular flagellated parasites characterised by the presence of a single mitochondrion, polycistronic transcription, *trans*-splicing and other unusual features (Maslov et al., 2019). The vast majority of the described taxa comprise monoxenous species, restricted to a single host. All dixenous representatives, i.e. those with two hosts in the life cycle (*Trypanosoma*, *Leishmania* and *Phytomonas*) have evolved independently from their monoxenous kin (Lukeš et al., 2014). Historically, the classification and, consequently, identification of trypanosomatids were based on cell morphology, life cycle, and host specificity (Vickerman, 1976; McGhee and Cosgrove, 1980; Votýpka et al., 2015). With the development of molecular methods, culture-independent PCR-based approaches have become useful and often indispensable tools for accurate assaying of trypanosomatid diversity (Westenberger et al., 2004; d'Avila-Levy et al., 2015; Borghesan et al., 2018; Spodareva et al., 2018). They not only eliminate inherent subjectivity of microscopic evaluations, but also address the issue of non- or hardly cultivatable species and mixed infections (Yurchenko et al., 2009; Tognazzo et al., 2012; Grybchuk-Ieremenko et al., 2014; Kostygov et al., 2014).

Examination of parasites from the dipteran and heteropteran hosts collected in various localities in Europe, Central and South America, sub-Saharan Africa, and China, including biodiversity hotspots, enabled detailed analyses of geographic distribution and diversity of trypanosomatids (Votýpka et al., 2012a, 2019; Týč et al., 2013). The average prevalence of infection in these regions ranged from 16% in China (Votýpka et al., 2010) to 26% in sub-Saharan Africa (Votýpka et al., 2012a) and 30% in the Neotropics (Maslov et al., 2010; Jirků et al., 2012). However, the means do not provide a complete picture, as some insect host species and/or genera were found frequently infected, while some were consistently free of parasites. For example, the detection of *Leptomonas pyrrhocoris*, TU***1***, (hereafter Typing Units (TUs) are used as proxies of species) in distant areas (Europe, Africa, Asia and the Neotropics) implies its cosmopolitan distribution (Votýpka et al., 2012b). This particular species was analysed further by whole-genome sequencing of 13 isolates from different localities worldwide, demonstrating that differences at the genomic level correlate with geographic pattern (Flegontov et al., 2016).

Host specificity of monoxenous trypanosomatids is another intriguing question (Maslov et al., 2013). The emerging picture is controversial and can be influenced by ecological factors such as hosts' feeding habits and preferences in social behaviour (Kozminsky et al., 2015), as well as incomplete sampling of hosts. Certain species of insect trypanosomatids can easily cross the borders between hosts' taxa and infect insects of several different families. On the other hand, some parasite species or even genera are restricted to particular hosts, as exemplified by *Blastocrithidia papi* specific to *Pyrrhocoris apterus* (Frolov et al., 2017, 2018), *Phytomonas nordicus* associated with *Troilus luridus* (Frolov et al., 2016) or *Blechomonas* and *Leishmania* spp. confined to fleas and sandflies, respectively (Votýpka et al., 2013; Akhoundi et al., 2016).

In this work, we analysed the collection of heteropteran hosts captured in PNG for the presence of trypanosomatids, the prevalence of infection and host specificity. In addition to discovering new parasite TUs, we recorded new insect hosts for the widespread trypanosomatid species and revealed potential cases of host-parasite coevolution.

2. Materials and methods

2.1. Field work and establishment of primary cultures

Insects of the suborder Heteroptera were collected during May 2011. Sampling was performed in two PNG provinces and eight localities – Madang Province: Nagada – The New Guinea Binatang Research Centre (5°9′23″S, 145°47′41″E, 20 m above sea level (masl)), Baitabag (5°8′46″S, 145°46′36″E, 40 masl), Ohu (5°14′1″S, 145°40′42″E, 215 masl), Mis (5°9′20″S, 145°45′49″E, 70 masl), and Karkar Island – Kulili Estates (4°31′25″S, 145°59′11″E, 20 masl); East Highlands Province: Goroka (6°4′44″S, 145°22′56″E, 1600 masl), Mt. Gahavisuka Provincial Park (6°2′2″S, 145°25′28″E, 2000 masl), and Kegsugl (5°49′52″S, 145°5′10″E, 2780 masl).

Insects were captured by net sweeping from vegetation or by light attraction. Within the following 12 h, heteropterans were killed and surface-sterilised with 70% ethanol, washed and dissected in 0.9% sterile saline solution as described previously (Yurchenko et al., 2016). Midgut, hindgut, and Malpighian tubes were squeezed separately with a cover slip and carefully examined for parasite infection using a portable microscope with 400× magnification as described elsewhere (Votýpka et al., 2013).

One aliquot of the positive material was transferred from the slide to 2% SDS, 100 mM EDTA solution for further DNA isolation and stored at room temperature in the field or −20 °C in the laboratory. The second half of the sample was inoculated into 1 ml of Brain Heart Infusion medium (Sigma-Aldrich, St. Louis, USA) supplemented with 10 μg/ml of hemin, 100 mg/ml of gentamicin, 1000 U/ml of penicillin and 1.5 mg/ml of fluorocytosine at room temperature as described previously (Votýpka et al., 2014; Kostygov et al., 2016).

2.2. Host insect identification

To facilitate host identification, most specimens were dry-mounted and deposited in the collections of the Department of Entomology, National Museum, Prague, Czechia. When appropriate, the material was sent for identification to specialists on particular groups (H. Brailovsky – Coreidae; P.-P. Chen and N. Nieser – Nepomorpha, Gerromorpha; F. Chérot – Miridae; E. Kondorosy – Lygaeoidea excl. Pachygronthidae; J. A. Lis – Cydnidae; D. Rédei – Scutelleridae; J. L. Stehlík – Pyrrhocoroidea; P. Štys – Colobathristidae). The remaining specimens were compared with available taxonomic revisions and/or the collections of The Natural History Museum, London, UK. Based on the available catalogues and revisions, the distribution of the particular species and genus group taxa of hosts was sorted into the following categories: ENDE = taxon endemic to New Guinea and the adjacent small islands; SUBE = taxon distributed to the east of Wallace's Line (in Oceania and Australian region); and WIDE = widely distributed taxa, including other biogeographic realms (Table 1, Supplementary Table S1).

Table 1
Summary of the trypanosomatid-positive insect host species and studied isolates of parasites from Papua New Guinea.

Host		Host distribution		Locality	Infection			Trypanosomatid		
Species	Stage	Species	Genus		Rate	Site	Intensity	Isolate	TU (SSU)	GenBank
Alydidae										
Leptocorisa acuta	Ad.	WIDE	WIDE	Baitabag	2/8	HG	+++	PNG 22	TU6/7C	MK929409
	Ad.					MG	++	PNG 23	TU6/7C	MK929410
	Ad.			Goroka	5/11	MG/HG	++	PNG 76	TU6/7C	MK929450
	Ad.					MG/HG	+++	PNG 77	**TU191**	KY593740
	Ad.					MG/HG	+++	PNG 78	**TU192**	MK929451
	Ad.					MG/HG	+	PNG 79	TU6/7C	MK929452
	Ad.					MG/HG	+++	PNG 80	TU6/7C	MF969020
	Ad.			Mt. Gahavisuka, Nagada	0/13					
Riptortus annulicornis	Ad.	WIDE	WIDE	Baitabag	1/1	HG	+++	PNG 123	**TU201**, **TU208**	MN215469, MN215470
	Ad.			Mis	9/14	MG	++	PNG 64	**TU201**	MF969019
	Ad.					MG	+++	PNG 65	TU6/7C	MK929441
	Ad.					HG	++	PNG 66	**TU201**	KY593737
	Ad.					HG	+++	PNG 105	**TU201**	MF969036
	Ad.					MG	+++	PNG 106	TU6/7C	MF969037
	Ad.					MG	++	PNG 107	**TU201**	MF969038
	Ad.					MG	+	PNG 108	**TU202**	KY593781
	Ad.					NA	+	PNG 109	TU6/7C	MK929456
	Ad.					MG	++	PNG 116	**TU201**	KY593787
	Ad.			Karkar, Nagada	0/10					
Riptortus linearis	Ad.	WIDE	WIDE	Nagada	3/10	MG	+++	PNG 17	TU6/7C	MK929406
	Ad.					MG	++	PNG 29	TU6/7C	MK929416
	Ad.					AMG	+++	PNG 30	TU6/7C	MK929417
	Ad.			Mis, Karkar	0/2					
Riptortus sp.	La.	?	WIDE	Mis	9/51	MG	+++	PNG 104	**TU201**	MF969035
	La.					MG	+	PNG 124	**TU201**	MF969040
	La.					MG	++	PNG 125	**TU201**	MF969041
	La.					MG	++	PNG 126	**TU201**	MF969042
	La.					MG	+++	PNG 127	**TU207**	MF969043
	La.					MG	+++	PNG 128	**TU201**, **TU208**	MF969044, MN215471
	La.					MG	+++	PNG 129	TU6/7C	KY593806
	La.					MG	++	PNG 130	**TU201**	KY593807
	La.					MG	+	PNG 131	**TU201**	MF969045
	La.			Karkar	0/3					
Belostomatidae										
Lethocerus insulanus	Ad.	WIDE	WIDE	Mis	1/1	NA	+	PNG 117	PCR-negative	
Coreidae										
Gralliclava irianensis	Ad.	SUBE	WIDE	Goroka	1/4	HG	++	PNG 81	**TU193**	MK929453
	Ad.			Mt. Gahavisuka	10/28	HG	++	PNG 84	**TU193**, **TU195**	MN215472, KY593749
	Ad.					MG	++++	PNG 85	TU63 (Ch7)	MK929454
	Ad.					MG	+	PNG 86	**TU193**	MF969022
	Ad.					MG	++	PNG 87	**TU193**	KY593752
	Ad.					MG	+	PNG 88	**TU193**, **TU195**	MN215473, MF969023
	Ad.					MG	+	PNG 89	**TU193**	MF969024
	Ad.					MG	+++	PNG 90	**TU193**	MF969025
	Ad.					MG	+	PNG 91	**TU193**	MF969026

(*continued on next page*)

Table 1 (*continued*)

Host		Host distribution		Locality	Infection			Trypanosomatid		
Species	Stage	Species	Genus		Rate	Site	Intensity	Isolate	TU (SSU)	GenBank
	Ad.					MG	+	PNG 92	***TU195***	MK929455
	Ad.					MG	++	PNG 93	***TU193***	MF969027
	Ad.			Nagada	0/19					
Plinachtus melinus	Ad.	SUBE	WIDE	Nagada	1/2	MG	++	PNG 68	***TU188***	MK929442
Gelastocoridae										
Nerthra conabilis	Ad.	ENDE	WIDE	Mt. Gahavisuka	2/31	AMG	++	PNG 82	***TU194***	MF969021
	Ad.					MG	+	PNG 83	***TU194***	KY593746
Gerridae										
Gerrinae gen. sp.	La.	?	?	Baitabag	1/1	MG	+++	PNG 24	TU*89*	MK929411
Limnometra cf. *kallisto*	La.	SUBE	WIDE	Baitabag	1/11	MG	++	PNG 20	PCR-negative	
Limnometra ciliata	Ad.	WIDE	WIDE	Nagada	2/3	MG	+++	PNG 33	TU*89*	MK929419
	Ad.					MG	+++	PNG 34	TU*89*	MK929420
Tenagogonus sp.[a]	Ad.	ENDE	WIDE	Baitabag	1/2	MG	+++	PNG 21	TU89	MK929408
Heterogastridae										
Parathyginus annulicornis	Ad.	ENDE	WIDE	Baitabag	1/2	MG	+++	PNG 115	***TU205***	MF969039
Largidae										
Delacampius lateralis	Ad.	SUBE	WIDE	Nagada	5/7	MG	+++	PNG 03	***TU174***	MF969016
	Ad.					MG	++	PNG 16	***TU210***	MK929405
	Ad.					MG	+++	PNG 54	***TU174***	MK929436
	Ad.					MG	+++	PNG 55	***TU174***	MK929437
	Ad.					MG	++	PNG 136	***TU210***	KY593813
	Ad.			Baitabag	1/1	MG	++	PNG 27	***TU210***	MK929414
	Ad.			Ohu	6/10	MG	+++	PNG 40	***TU174***	MK929425
	Ad.					MG	+++	PNG 41	***TU174***	MK929426
	Ad.					MG	+++	PNG 42	***TU174***	MK929427
	Ad.					MG	+	PNG 47	***TU183***	MK929430
	Ad.					MG	++	PNG 48	***TU210***	MK929431
Lygaeidae										
Graptostethus servus	Ad.	WIDE	WIDE	Nagada	1/1	MT	++	PNG 74	***TU187***	MK929448
Thunbergia torrida	Ad.	SUBE	WIDE	Nagada	4/23	MG	+	PNG 04	***TU175***	MK929397
	Ad.					MT	+++	PNG 50	***TU184***	KY593731
	Ad.					MT	+++	PNG 51	***TU184***	MK929433
	Ad.					MT	+++	PNG 49	***TU184***	MK929432
Miridae										
Chaetedus rutilans	Ad.	ENDE	SUBE	Mt. Gahavisuka	1/1	NA	++	PNG 99	***TU199***	MF969032
	Ad.			Kegsugl	1/21	MG	++	PNG 100	TU*247*	KY593769
	Ad.			Goroka	0/2					
Lasiomiris albopilosus	Ad.	WIDE	WIDE	Mt. Gahavisuka	3/8	HG	++	PNG 94	***TU199***	MF969028
	Ad.					NA	++	PNG 97	***TU199***	MF969030
	Ad.					NA	+	PNG 98	***TU200***	MF969031
	Ad.			Kegsugl	1/1	MG	+++	PNG 101	***TU199***	KY593770
	Ad.			Goroka	2/6	MG	++	PNG 102	***TU199***	MF969033
	Ad.					MG	++	PNG 103	***TU199***	MF969034
Pentatomidae										
Alciphron glaucus	Ad.	WIDE	WIDE	Nagada	1/2	MG	+++	PNG 02	***TU173***	KY593709
Antestia semiviridis	Ad.	WIDE	WIDE	Nagada	4/10	NA	+	PNG 01	TU*44*	MK929396
	Ad.					MG	+	PNG 05	***TU176***	MK929398
	Ad.					NA	+	PNG 07	PCR-negative	
	Ad.					TMG	++	PNG 08	TU*44* (Ch1)	KY593713
	Ad.			Ohu	4/28	MG	+++	PNG 35	TU*44*	MK929421
	Ad.					MG	+++	PNG 36	TU*44*	MK929422
	Ad.					MG	+	PNG 37	TU*44*	MK929423
	Ad.					MG	+++	PNG 46	TU*44*	MK929429
Eysarcoris cf. *trimaculatus*	Ad.	SUBE	WIDE	Baitabag	1/3	NA	+	PNG 25	TU*77*	MK929412
	Ad.			Nagada	2/9	TMG	++	PNG 09	***TU177***	MK929400
	Ad.					MG	++	PNG 28	TU*77*	MK929415
Pyrrhocoridae										
Antilochus reflexus	Ad.	SUBE	WIDE	Mis	1/1	MG	+++	PNG 57	***TU186***	KY593733
Dindymus pyrochroa	Ad.	SUBE	WIDE	Mis	1/5	MG	++++	PNG 58	TU*63*	MK929438
Dysdercus fuscomaculatus	Ad.	WIDE	WIDE	Nagada	3/10	MG	+	PNG 18	***TU181***	MF969017
	Ad.					MG	+++	PNG 31	***TU181***	KY593722
	Ad.					MG	++	PNG 138	***TU181***	MF969047
	Ad.			Ohu	2/4	AMG	+++	PNG 38	***TU181***	KY593727
	Ad.					NA	++	PNG 45	***TU181***	MF969018

Table 1 (*continued*)

Host		Host distribution		Locality	Infection			Trypanosomatid		
Species	Stage	Species	Genus		Rate	Site	Intensity	Isolate	TU (SSU)	GenBank
Dysdercus cf. cingulatus	Ad.	WIDE	WIDE	Nagada	1/1	MG	+++	PNG 56	TU*63*	KY593732
Paraectatops ruficosta	Ad.	ENDE	SUBE	Nagada	3/15	NA	+	PNG 32	TU*63*	MK929418
ruficosta	Ad.					NA	+	PNG 70	TU*63*	MK929444
	Ad.					MG	++	PNG 73	TU*63*	MK929447
Reduviidae										
Helonotus cf. *sexspinosus*	Ad.	SUBE	WIDE	Mis	2/3	AMG	+++	PNG 61	TU*63*	MK929440
	Ad.					MG	++++	PNG 133	***TU209***	KY593810
Helonotus sp. 1	Ad.	ENDE	WIDE	Mis	2/8	NA	+	PNG 63	PCR-negative	
	Ad.					NA	+	PNG 62	***TU187***	KY593735
Helonotus sp. 2	Ad.	ENDE	WIDE	Mis	1/2	MG	++++	PNG 135	***TU209***	MF969046
Helonotus sp. 3	Ad.	ENDE	WIDE	Mis	1/1	MG	++++	PNG 134	TU*63*	MK929465
Helonotus sp. 4	Ad.	ENDE	WIDE	Mis	1/1	MG	++++	PNG 132	***TU209***	KY593809
Paloptus sp.	Ad.	ENDE	ENDE	Mis	1/4	MG	+++	PNG 59	TU*63*	MK929439
Paloptus annulatus	Ad.	ENDE	ENDE	Baitabag	1/2	NA	+	PNG 26	TU*89*	MK929413
Pristhesancus sp.	Ad.	ENDE	WIDE	Mis	1/1	HG	++	PNG 60	TU*83*	KY593734
Rhyparochromidae										
Gyndes novaeguineae	Ad.	ENDE	WIDE	Mt. Gahavisuka	2/15	NA	+	PNG 95	***TU196*, *TU197***	MN215474, KY593764
	Ad.					NA	++	PNG 96	***TU198***	MF969029
	Ad.			Goroka	0/2					
Gyndes papuaguineae	Ad.	ENDE	WIDE	Nagada	2/3	HG	++	PNG 52	***TU189***	MK929434
	Ad.					MT	+++	PNG 72	***TU189***	MK929446
Gyndes sp.[a]	Ad.	ENDE	WIDE	Goroka	1/2	HG	++++	PNG 75	***TU190***	MK929449
Horridipamera nietneri	Ad.	WIDE	WIDE	Nagada	12/	NA	++	PNG 06	***TU206***	MK929399
	Ad.				53	MG	+	PNG 14	***TU206***	MK929403
	Ad.					MT	+	PNG 15	***TU206***	MK929404
	Ad.					MG	+++	PNG 19	***TU206***	MK929407
	Ad.					MT	+++	PNG 71	***TU206***	MK929445
	Ad.					NA	++	PNG 111	***TU206***	MK929458
	Ad.					HG	++	PNG 118	***TU206***	MK929460
	Ad.					MG	+++	PNG 119	***TU206***	MK929461
	Ad.					MG	+++	PNG 120	***TU206***	MK929462
	Ad.					HG	+++	PNG 121	***TU206***	MK929463
	Ad.					NA	+++	PNG 122	***TU206***	MK929464
	Ad.					MG	++	PNG 137	***TU206***	MK929466
Kanigara fumosa	Ad.	ENDE	WIDE	Nagada	2/4	MG	+++	PNG 12	***TU178***	KY593715
	Ad.					MG	+	PNG 13	***TU179*, *TU180***	KY593716, KY593717
	Ad.			Baitabag	0/1					
Aristaenetus diabolicus	Ad.	ENDE	ENDE	Baitabag	1/3	NA	++	PNG 112	***TU203***	KY593784
Narbo biplagiatus	Ad.	WIDE	WIDE	Baitabag	1/1	NA	+	PNG 114	***TU204***	MK929459
Neolethaeus cf. *cantrelli*	Ad.	SUBE	WIDE	Ohu	1/2	NA	+	PNG 43	***TU182***	KY593729
	Ad.			Baitabag	1/1	MG	+	PNG 113	***TU1***	KY593785
	Ad.			Nagada	0/4					
Pamerana sp.[a]	Ad.	ENDE	WIDE	Nagada	1/2	NA	++	PNG 53	***TU185***	MK929435
Scutelleridae										
Calliphara regalis	Ad.	SUBE	WIDE	Nagada	4/9	TMG	+++	PNG 10	TU*44*	MK929401
	Ad.					TMG	++++	PNG 11	TU*44*	MK929402
	Ad.					MG	++	PNG 69	TU*44*	MK929443
	Ad.					MG	+++	PNG 110	TU*44*	MK929457
Coleotichus biroi	Ad.	ENDE	WIDE	Ohu	2/2	MG	++	PNG 39	TU*44*	MK929424
	Ad.					MG	+++	PNG 44	TU*44*	MK929428

Ad, adult; La, larvae; AMG, abdominal midgut; HG, hindgut; MG, midgut; MT, Malpighian tubules; TMG, thoracic midgut; NA, not available. Distribution of the particular species- and genus-group taxa of hosts is sorted to the following categories: ENDE, taxon endemic to New Guinea and the most close off-shore islands; SUBE, taxon distributed in Australian Region east of Wallace line; and WIDE, widely distributed taxa. Typing Units (TUs) in bold were not documented before. [a]New (undescribed) species of insects.

2.3. DNA isolation, PCR amplification, cloning and sequencing

Total DNA was isolated from the preserved infected field samples by a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The DNA was used for amplification of the 18S rRNA gene with either primers 1127F and 1958R (generating ~ 900 bp fragment) or primers S762 and S763 (producing an almost full-length gene, ~2.1 kb) as described previously (Maslov et al., 1996; Kostygov and Frolov, 2007). When amplification with the second primer pair resulted in a very low PCR product concentration, a second round of PCR was performed with nested primers TRnSSU-F2 and TRn-SSU-R2 (Seward et al., 2017). In the case of mixed infections, PCR products were cloned using the InsTA PCR Cloning Kit (Thermo Fischer Sci., Waltham, USA) and several clones were sequenced. Sequencing of the short 18S rRNA fragment was performed using the amplification primers, whereas the long amplicons were sequenced with primers 883F, 908R, S757 and A757 as described elsewhere (Kostygov et al., 2011; Gerasimov et al., 2012).

2.4. Phylogenetic analysis

In total, 185 18S rRNA sequences (147 retrieved from GenBank and 38 representing TUs unique for PNG) were aligned using MAFFT v. 7.4 (Katoh and Standley, 2013). Alignment trimming was not performed in order to preserve differences between closely related species. This alignment is available from MendeleyData via the link https://doi.org/10.17632/7smx5bgr63.2. Maximum likelihood phylogenetic inference was performed using IQ-TREE software (Nguyen et al., 2015) with the TIMe + I + G4 model selected under Bayesian information criterion by the built-in ModelFinder (Kalyaanamoorthy et al., 2017). Branch supports were assessed by ultrafast bootstrapping with 1000 replicates (Minh et al., 2013).

3. Results and discussion

3.1. Field examination of heteropteran hosts

Heteropteran insects of 23 different families and 138 species from eight localities in PNG were examined for the presence of trypanosomatid parasites (Table 1, Supplementary Table S1). Out of 907 dissected and analysed specimens, 137 (belonging to 45 species) were found to be infected by trypanosomatids, with the average prevalence of infection being 15% (Table 1, Supplementary Table S1). It was also calculated for host families with 20 or more analysed representatives. The highest prevalence of infection was documented in the Pyrrhocoridae (11 positives out of 40 examined, 27.5%), followed by the Reduviidae (10/44, 23%), Alydidae (29/126, 23%), Lygaeidae (5/26, 19%), Gerridae (5/30, 17%), Rhyparochromidae (24/153, 16%), Pentatomidae (12/81, 15%), Miridae (8/64, 12.5%), Coreidae (12/133, 9%), and Gelastocoridae (2/32, 6%). Representatives of the families Colobathristidae, Cydnidae and Tessaratomidae (40, 50 and 46 specimens analysed, respectively) were not infected. The observed infection intensity varied from very mild to heavy. Although in some dissected specimens it was not possible to unambiguously determine localization of parasites, we concluded that flagellates were predominately found in the midgut (69%), followed by the hindgut (12%), and the Malpighian tubules (5%) (Table 1).

The uneven distribution of trypanosomatids over heteropteran taxa is determined by many factors. For example, predatory bugs may get some additional parasites from their prey. In our data, these were Reduviidae, Gerridae, and Gelastocoridae. Gerridae are also permanently associated with water, and this may facilitate the survival of infective stages of parasites in the environment (Schuh and Slater, 1995). Sap-sucking bugs such as Pentatomidae, Coreidae, and Alydidae can obtain Phytomonas spp. from infected plants (Camargo et al., 1990). The parasites' absence in the specimens of the family Tessaratomidae can be explained by the fact that these insects feed on plants of the orders Rosales and Sapindales (Schuh and Slater, 1995), in which no species susceptible to trypanosomatids are known (Podlipaev, 1990).

The majority of the bugs belonging to the family Rhyparochromidae, as well as some members of Lygaeidae and Pyrrhocoridae, spend most of their time on the ground looking for seeds (Schaefer and Panizzi, 2000). Food on the ground, compared with plants' surfaces, has a higher probability of being contaminated with insects' faeces containing infective stages of parasites. Various true bugs, in general, occasionally practise coprophagy or necrophagy, but in Rhyparochromidae and Pyrrhocoridae this behaviour is especially frequent as judged by numerous records (Schuh and Slater, 1995). In addition, many species of these two families, as well as some Lygaeidae, are gregarious. All these factors have been experimentally demonstrated to be responsible for high infection rates in the firebug *Pyrrhocoris apterus* (Frolov et al., 2017). Coprophagy and high abundance of some Miridae apparently increase these bugs' chances of obtaining trypanosomatids (Schaefer and Panizzi, 2000). We suggest that such factors as co-occurrence of susceptible hosts, as well as physiology and lifespan of insects, may also influence distribution of the parasites across host taxa.

3.2. Phylogenetic analysis

Out of 137 samples from the infected bugs, the 18S rRNA gene was amplified from 133. In most cases, we were able to obtain nearly full-length sequences. Based on these data, 46 TUs were documented including 38 new and eight previously recorded ones (Fig. 1). In six specimens, simultaneous infection by two trypanosomatid species was documented based on the sequencing data (Table 1).

For each of the major known clades of trypanosomatids, reference sequences were selected, whereas for the clades/genera comprising TUs, found in the PNG dataset, all available 18S rRNA gene

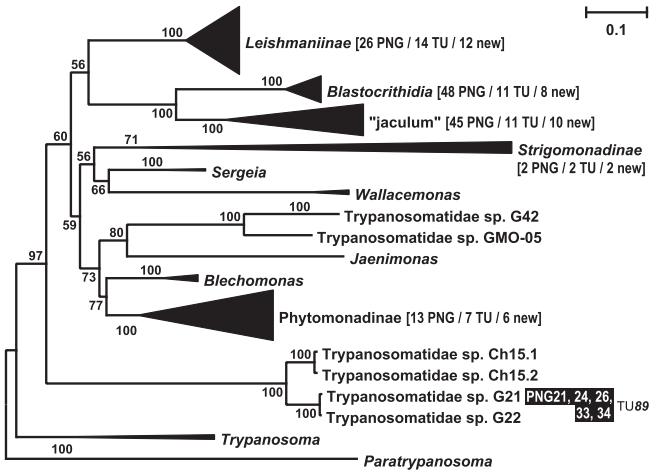

Fig. 1. Summarised 18S rRNA maximum likelihood phylogenetic tree of the family Trypanosomatidae. Most genus or subfamily level clades are collapsed. Their contents are shown in individual subtrees (Figs. 2–5). Numbers in parentheses show the total number of obtained Papua New Guinean sequences, the total number of Typing Units and the number of new TUs for a respective clade. The ultrafast bootstrap values over 50% (1000 replicates) are shown at the nodes. The tree was rooted with the sequence of *Paratrypanosoma confusum*. The scale bar denotes the number of substitutions per site. PNG isolates are highlighted.

sequences were used. The resulting tree topology appeared congruent with that published previously (Kostygov et al., 2016; Yurchenko et al., 2016; Frolov et al., 2017; Ishemgulova et al., 2017) and all main clades were well supported (Fig. 1).

Most new TUs clustered within the subfamilies Leishmaniinae and Phytomonadinae, the "*jaculum*" group, and the genus *Blastocrithidia* similarly to the recent study of trypanosomatid biodiversity in Neotropics (Kozminsky et al., 2015). Known species and/or TUs were also mainly distributed over these four groups. In addition, two new TUs were associated with the subfamily Strigomonadinae and one previously recorded TU was revealed in an unnamed lineage (Fig. 1).

Leishmaniinae lead both in the total number of TUs (14) and in the number of the new ones (12), although these originated from only 26 samples. One of the previously documented species was widely distributed *Leptomonas pyrrhocoris* (TU***1***), represented here by a single isolate PNG 113 (Fig. 2). Similarly, another previously documented species was *Crithidia* sp. G15 (TU***83***), also found in a single specimen PNG 60 (Fig. 2). This trypanosomatid was originally classified as *Crithidia otongatchiensis* (Yurchenko et al., 2014), but later demonstrated to differ from that species by 18S rRNA and glycosomal glyceraldehyde-3-phosphate dehydrogenase (gGAPDH) sequences. Moreover, the two species bear distinct RNA viruses (Grybchuk et al., 2018). New TUs within the subfamily Leishmaniinae were spread over the clade. The most interesting

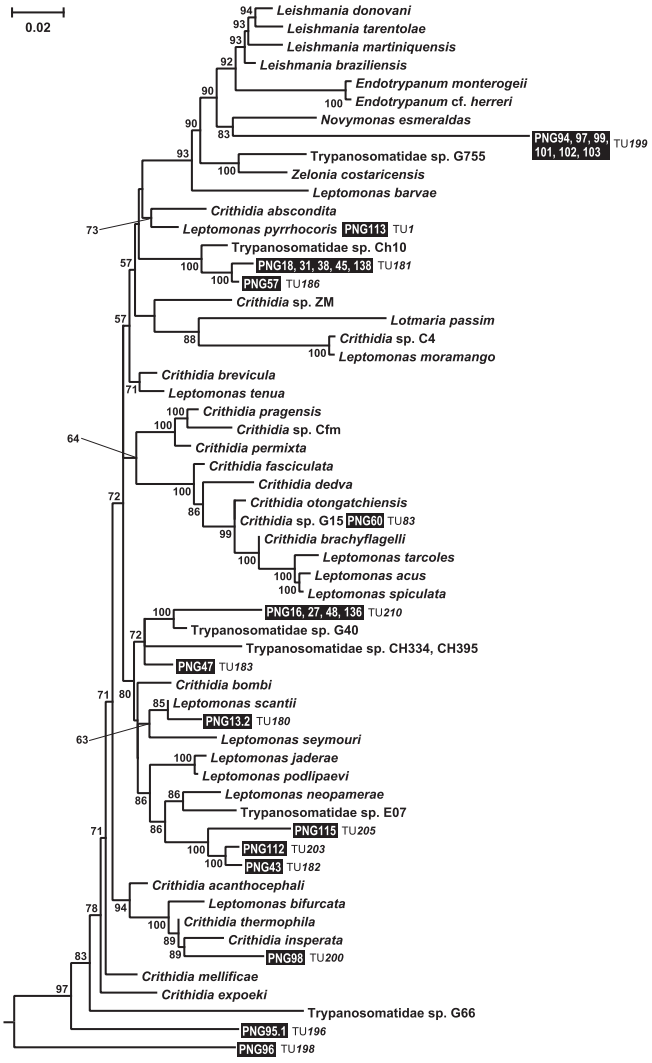

Fig. 2. Expanded subtree of the subfamily Leishmaniinae.

of them were TU***199*** (PNG 94, 97, 99, 101–103), constituting a long branch that clusters with the endosymbiont–bearing species *Novymonas esmeraldas*, as well as TU***196*** and TU***198*** appearing as the earliest branches within the subfamily.

Eleven *Blastocrithidia* TUs were identified in the PNG collection and eight of them were new. Out of 48 sequences falling into this clade, one quarter belonged to TU***44*** (Fig. 3). These were obtained from three heteropteran species (Table 1). TU***63***, which was also recorded elsewhere, demonstrated relatively high frequency (nine specimens) belonging to five different host species (Fig. 3; Table 1). The third previously documented species (TU***247***), found earlier in *Lygus* sp. from Russia and *Lygus hesperus* from the USA (Supplementary Table S2), was revealed in a single specimen (Fig. 3, Table 1). This undescribed trypanosomatid was shown to have a non-canonical genetic code (Zahonova et al., 2016). One of the new TUs was represented by a large number of sequences, but they all originated from a single host species (Table 1), which was examined quite intensely (53 specimens). In general, the revealed TUs were not associated with a particular subclade within the *Blastocrithidia* lineage, but four new TUs grouped with the Chinese isolate Ch5 (Fig. 3).

The yet formally undescribed "*jaculum* group" contained 11 TUs from PNG, all of which but one were new. The TU***6/7C*** previously recorded in many countries throughout the world was represented by 12 out of 45 total sequences falling into this clade (Fig. 3, Table 1). These sequences originated from four bug species. One of the new TUs was even more frequent, with 13 sequences obtained from two host species (Fig. 3, Table 1). The distribution of TUs from PNG was uneven, with the majority of them (TU***6/7C***, ***176***, ***179***, ***184***, ***201***, ***202***, ***207***) being concentrated within one subclade with a shallow branching pattern (Fig. 3).

Within the subfamily Phytomonadinae, six TUs were associated with the genus *Phytomonas* and one with *Herpetomonas*. The latter (TU***209***) was situated in a cluster of closely related *Herpetomonas* spp. (including the described species *Herpetomonas nabiculae* and *Herpetomonas samueli*) characterised by short branch lengths (Fig. 4). Three sequences from three *Helonotus* spp. represented this TU (Table 1). Only one previously recorded TU (TU***77***) fell into the genus *Phytomonas* (Fig. 4). It was found in two individuals of the same shield bug species (Table 1). The sample PNG 09 produced a quite similar, yet distinct, sequence assigned to the new TU***177*** (Fig. 3). The isolates PNG 02 (TU***173***) and PNG 68 (TU***188***) were very closely related to *Phytomonas francai* and *Phytomonas lipae*, respectively (Fig. 4). Two new TUs clustered with *Phytomonas oxycareni* and an unnamed species recently documented in Curaçao (isolate CC-83). This cluster represents the earliest branch of the genus *Phytomonas* known to date (Fig. 3).

Each of the two TUs falling into the subfamily Strigomonadinae were represented by a single isolate, both associated with the genus *Strigomonas* (Fig. 5). However, while PNG 77 (TU***191***) was closely related to *Strigomonas galati*, the PNG 95.2 (TU***197***) sequence was a sister to all *Strigomonas* spp., but separated from them by a considerable distance. Thus, it is unclear whether this TU should be assigned to *Strigomonas* or represents a new genus. One of the TUs (TU***89***) documented in PNG is a member of an anonymous trypanosomatid group, which is known only by 18S rRNA sequences. Five sequences obtained from four different heteropteran species belonged to this TU.

3.3. Host-parasite specificity and endemism

Out of eight TUs found elsewhere, four (TU***01***, TU***77***, TU***83*** and TU***247***) have been documented within this study only in the endemic (New Guinea) and sub-endemic (Australian region) heteropteran hosts (Table 1). This is counterintuitive, since there was no other way for these trypanosomatids to appear in PNG than

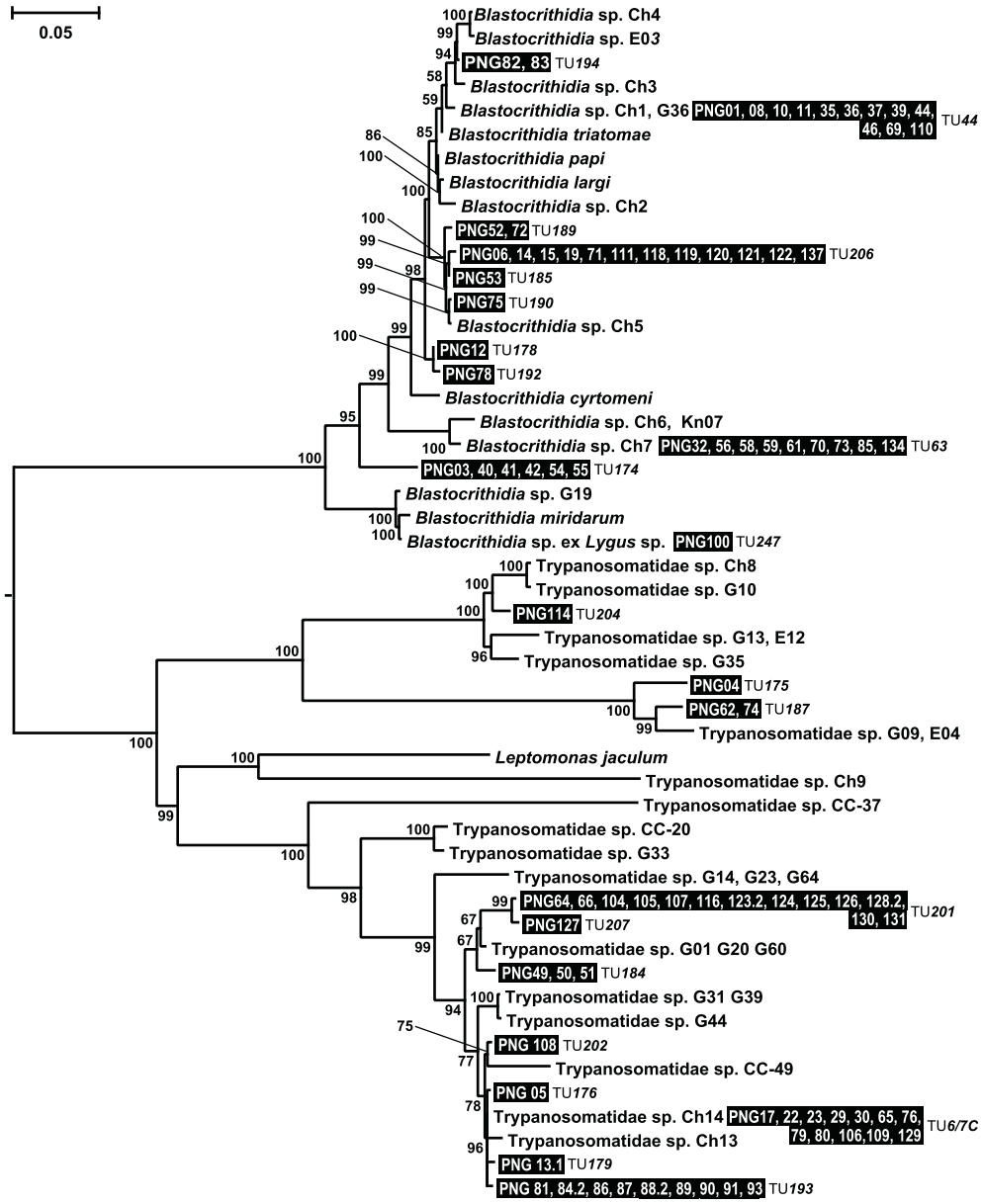

Fig. 3. Expanded subtree with the genus *Blastocrithidia* and "*jaculum*" phylogroup.

with widespread insect species. Apparently our sampling was not comprehensive enough to detect these TUs in such true bugs.

The situation with *Leptomonas pyrrhocoris* (TU***01***) is the most enigmatic (Fig. 2). This species has been regularly detected in various representatives of the family Pyrrhocoridae (mainly in the genera *Pyrrhocoris* and *Dysdercus*) from many countries all over the world (Supplementary Table S2), while in PNG it was found in a single individual of *Neolethaeus* cf. *cantrelli* (PNG 113, Rhyparochromidae). Together with the low intensity of the infection (Table 1) this suggests that this might be a non-specific infection. Indeed, this heteropteran species is considered to be a seed-predator with occasional sucking on dead insects. Thus, it could have obtained this parasite while feeding on a corpse of a pyrrhocorid bug. However, it is unclear why *L. pyrrhocoris* was not found in any of the forty examined specimens of the family Pyrrhocoridae belonging to seven species including three species of the genus *Dysdercus* (Table 1, Supplementary Table S1).

The case of TU***77*** is more understandable (Fig. 4). In agreement with its affiliation to the plant-parasitizing genus *Phytomonas*, this trypanosomatid has been previously reported from Ghana (Votýpka et al., 2012a) in various phytophagous bugs of the families Alydidae, Coreidae, Lygaeidae and Pentatomidae (Supplementary Table S2), suggesting its wide host specificity. In addition, there was a single record from a predatory bug of the family Reduviidae, undoubtedly representing a non-specific infection. In the PNG collection, this TU was detected in a sub-endemic species belonging to *Eysarcoris* (Pentatomidae), one of the previously listed host genera (Supplementary Table S2). Given its supposedly wide specificity, this parasite may inhabit un-sampled species of phytophagous heteropterans in PNG.

Crithidia sp. TU***83*** was detected in a single specimen of endemic *Pristhesancus* sp. belonging to the family Reduviidae (Fig. 2). Members of this family are prone to non-specific trypanosomatid infections (Kozminsky et al., 2015). Of note, a previous record of this trypanosomatid also came from a reduviid bug *Rhynocoris rapax*, captured in Ghana (Suppl. Table 2). Taking into account the predatory nature of these bugs, it is plausible that in both cases infections were non-specific.

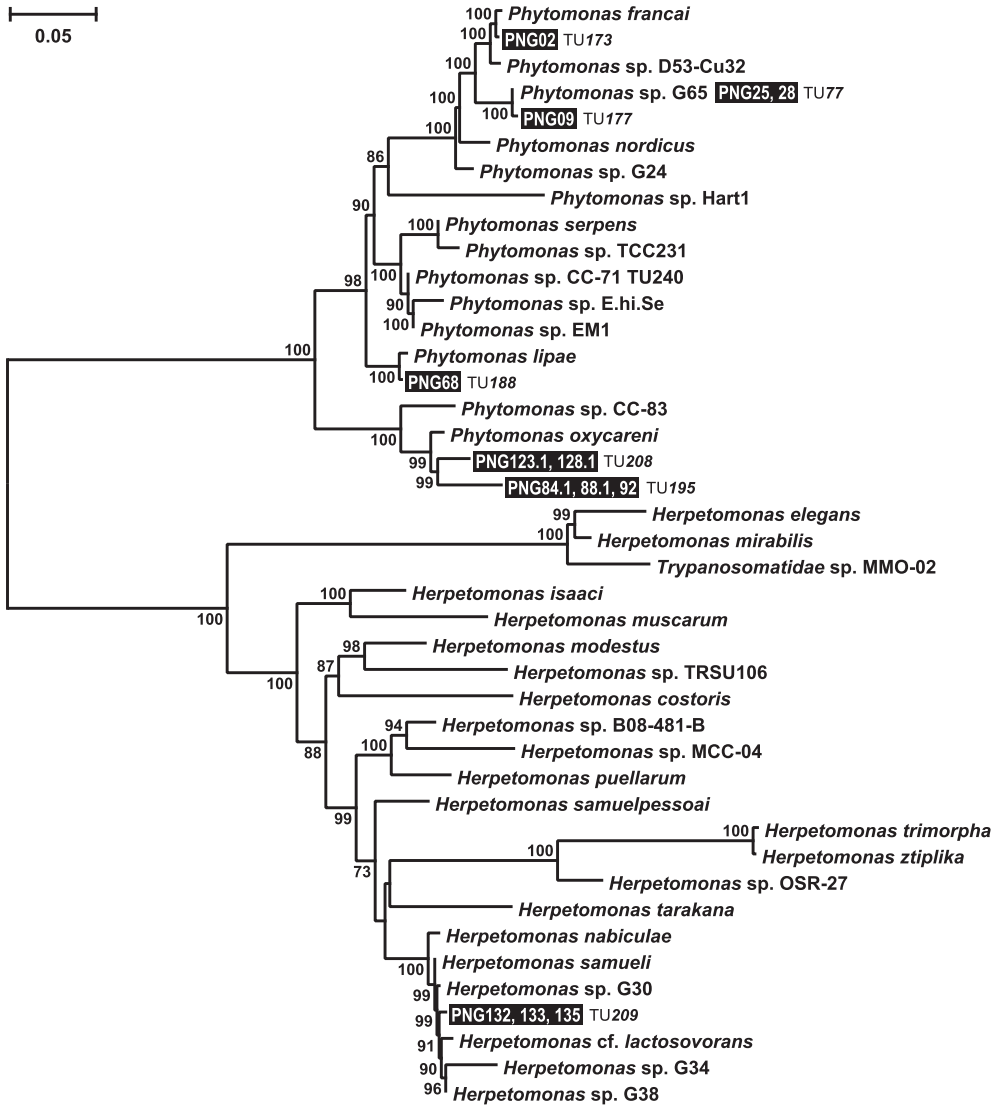

Fig. 4. Expanded subtree of the subfamily Phytomonadinae.

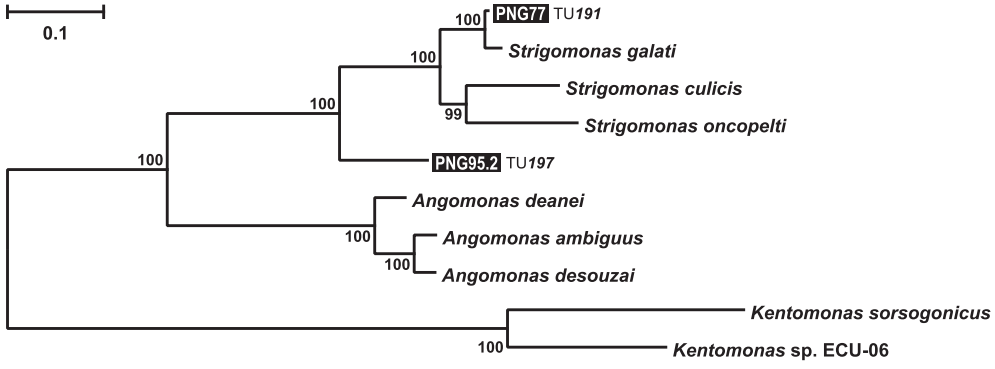

Fig. 5. Expanded subtree of the subfamily Strigomonadinae.

In the case of *Blastocrithidia* sp. TU***247*** (Fig. 3) the fact that it was documented only in one out of 22 examined individuals of *Chaetedus rutilans* may indicate that this was an occasional non-specific infection, although the host belongs to the same family, Miridae (Supplementary Table S2), as *Lygus* spp. in the previous records from Russia and the United States (Zahonova et al., 2016). We examined 19 species of this species-rich family, including some widespread ones, however for the majority of them only one to three specimens were dissected and none of these species belonged to *Lygus* sensu lato.

For trypanosomatids with wide ranges of known hosts, it is difficult to discriminate between specific and non-specific infections. However, a comparison of these flagellates' occurrences in different geographic regions together with prevalence and infection

intensity can help in understanding specificity of particular host-parasite associations. *Blastocrithidia* sp. TU***44*** (Fig. 3) has been previously found in 13 species of the families Alydidae, Coreidae, Geocoridae, Miridae, Pentatomidae, Pyrrhocoridae, Reduviidae, and Scutelleridae on different continents (Supplementary Table S2). Although the data unambiguously point to low host specificity of this TU, it is unlikely that all these recorded hosts were specific. In the PNG collection, this TU was detected in one species of the family Pentatomidae and two species of the related family Scutelleridae, thereby increasing the counts of recorded host species from both families and indicating that these infections should be specific. This is further supported by the observation that for each of the three species, the infections were non-unique and reached high intensity.

TU***6/7C*** of the "jaculum" phylogroup (Fig. 3) was previously documented in six species of Alydidae and two species of Reduviidae on different continents (Supplementary Table S2) with an apparent predominance of representatives from the first family. The PNG dataset supports this trend with all four revealed host species being members of the Alydidae. As in the case of TU***44***, these infections reached high intensity and, except for one species (*Riptortus* sp.), for which only larvae were examined, were not unique (Table 1). Thus, true bugs of the family Alydidae appear to be specific hosts of TU***6/7C***.

In the case of TU***89*** belonging to the unnamed clade (Fig. 1), the newly obtained data expand the range of involved hosts. Previously, this trypanosomatid was found in water striders (Gerridae) *Limnogonus hypoleucus* and *Tenagogonus albovittatus* from Ghana (Supplementary Table S2), while in PNG it was detected in three other species of the same family: *Limnometra ciliata*, an undescribed *Tenagogonus* sp. and one nymph of an unidentified species. In all these cases the intensity of infection was high, although the limited number of examined specimens does not allow judgement of their prevalence (Table 1). In addition, this TU was detected in a single reduviid *Paloptus annulatus* (PNG 26) with a mild infection, suggesting a non-specific host-parasite association. The available information on TU***89*** implies that this trypanosomatid has wide host specificity restricted to the family Gerridae, more specifically to the tribe Gerrini, to which all currently recorded hosts belong.

The situation with the host distribution for TU***63*** (Fig. 3) is unclear. Previously, it was recorded in five species from the families Belostomatidae, Coreidae, Gerridae, and Pentatomidae in China (Supplementary Table S2). In PNG it was found in one species of Coreidae, three species of Pyrrhocoridae and three species of Reduviidae. A simple comparison of the two host lists shows that only Coreidae is present in both of them. Indeed, among the Chinese samples the infection intensity was strong only in *Ochrochira* sp. (Coreidae), while in other species it was low (Votýpka et al., 2010). However, among the respective PNG samples only those from *Paraectatops ruficosta* (Pyrrhocoridae) had low or medium intensity, while in all other species it was high to very high (Table 1). Although it cannot be excluded that all three reduviid specimens belonging to three different species of two genera were caught soon after feeding on heavily infected prey, it is more plausible that the trypanosomatid in question has a very wide specificity, covering hosts from unrelated heteropteran families.

The majority of new TUs (33/38; 87%) were recorded only in one heteropteran species (Supplementary Table S2). That could be interpreted as their having high host specificity, but 24 of these TUs were documented only from a single individual, making this conclusion premature. Five TUs, which were identified in more than one host species, also demonstrate some specificity. TU***199*** from the subfamily Leishmaniinae (Fig. 2) was found in *Chaetedus rutilans* and *Lasiomiris albopilosus*, both from the same grass-feeding tribe Stenodemini (Miridae). TU***201*** ("*jaculum*" phylogroup, Fig. 3) and *Phytomonas* sp. TU***208*** (Fig. 4) were found in the same two species of *Riptortus* (Alydidae). *Herpetomonas* sp. TU***209*** (Fig. 4) was detected in three specimens each belonging to a different species of *Helonotus* (Reduviidae). Given the predatory nature of the hosts and affiliation of this TU to the genus *Herpetomonas*, usually associated with dipterans (Borghesan et al., 2013), these cases could be regarded as non-specific infections. However, all three *Helonotus* spp. specimens were heavily infected (Table 1) and the subclade enclosing TU***209*** consists exclusively of species/TUs isolated from heteropterans, mostly predatory ones: *Herpetomonas* spp. G30 and G38 from *Coranus* sp. (Reduviidae), *Herpetomonas* sp. G34 from *Rhynocoris albipilosus* (Reduviidae), *H. nabiculae* from *Nabis flavomarginatus* (Nabidae), *H. samueli* from *Zelus leucogrammus* (Reduviidae) and *Herpetomonas* cf. *lactosovorans* from phytophagous *Pachygronta barberi* (Lygaeidae) (Yurchenko et al., 2009; Kostygov et al., 2011; Votýpka et al., 2012a; Borghesan et al., 2013). Since at least one of them (*H. nabiculae*) was shown to specifically develop in its host (Frolov and Skarlato, 1995), the specificity of TU***209*** to *Helonotus* spp. is also very likely. TU***187*** of the "*jaculum*" phylogroup (Fig. 3) caused medium and weak infections in single specimens of *Graptostethus servus* (Lygaeidae) and *Helonotus* sp. (Reduviidae), respectively. Its location in the Malpighian tubules of the lygaeid host argues for its specificity for this insect.

Out of 38 new (potentially endemic) TUs, 13 were found in widely distributed and 24 in endemic or sub-endemic heteropteran host species. Such distribution suggests intensive radiation of trypanosomatids in this previously unsampled region. The percentage of the infected species differs between endemic (15 and 9.4%, hereafter numbers refer to levels of species and genera, respectively), sub-endemic (32.5 and 8.5%) and widespread (38.2 and 25.5%) species. This clearly points to higher infection rates for widely distributed host taxa. However, given the large diversity of host species it is also possible that these findings are burdened by a sampling bias (for example, endemic or sub-endemic versus widespread), and for a more precise assessment, a significantly higher number of host species and specimens would have to be analysed.

3.4. Potential host-parasite coevolution

The increasing number of TUs described to date allowed us to compare phylogeny and distribution of trypanosomatids over a wide range of host taxa, thereby addressing the extent of coevolution. Although the absence of reliable phylogenetic inferences for the heteropteran taxa precluded thorough comparison of hosts' and parasites' phylogenies side by side, it was obvious that several clades are associated with particular host groups. For example, within the subfamily Leishmaniinae, there is a cluster consisting of TU***66*** (represented by Ch10), TU***186*** and TU***181*** (Fig. 2) from *Melamphaus faber*, *Antilochus reflexus*, and *Dysdercus fuscomaculatus*, respectively, which all belong to the family Pyrrhocoridae. Another such case in the same subfamily is represented by a clade comprising TU***182***, TU***203***, and TU***205*** (Fig. 2) from *Neolethaeus* cf. *cantrelli*, *Aristaeneus diabolicus*, and *Parathyginus annulicornis*, respectively, which are all members of the superfamily Lygaeoidea. Importantly, the first two TUs are sister to each other and have "cousin" relationships with the third one. In agreement with this, the hosts of TU***182***, TU***203*** are members of the family Rhyparochromidae, while the third host belongs to a closely related, but separate, family Heterogastridae.

In *Blastocrithidia* (Fig. 3), we revealed a new clade of closely related species represented by TU***185*** from *Pamerana* sp., TU***189*** from *Gyndes papuaguineae*, TU***190*** from *Gyndes* sp., and TU***206*** from *Horridipamera nietneri* as well as the Chinese isolate Ch5 (TU***14***) from *Gyndes* sp. All these host species belong to the family Rhyparochromidae.

Finally, in the "*jaculum*" phylogroup (Fig. 3), we identified a clade formed by trypanosomatids parasitizing Lygaeidae: TU***175*** from *Thunbergia torrida*, TU***187*** from *Graptostethus servus*, as well as TU***88*** represented by the African isolates G09 and E04 from *Aspilocoryphus fasciativentris* and *Spilostethus pandurus*, respectively.

It cannot be excluded that some or all of the above-described patterns of coevolutionary events are consequences of a similar physiology of true bugs of a particular genus or family. Such a similarity would facilitate horizontal transitions of parasites between hosts, which would be difficult to distinguish from genuine coevolution. Interestingly, none of the discussed examples was restricted to endemic host genera, therefore if coevolution really occured, it was not limited to the area of PNG and accompanied by bugs' dispersal.

4. Conclusions

In this work we surveyed trypanosomatids from heteropteran hosts collected in Papua New Guinea. This region is a well-known biodiversity hotspot for macro-organisms, among which insects represent the majority (Marshall and Beehler, 2007). Therefore, we anticipated documenting not only a corresponding high diversity, but also a high proportion of novel parasitic trypanosomatids. Our expectations were fulfilled: out of 907 specimens belonging to 138 species, 103 (sub)genera and 23 different families of Heteroptera collected in eight localities, 38 new trypanosomatid TUs were identified. The remaining eight TUs were detected mostly in hosts with cosmopolitan distribution. The proportion of novel TUs (83%) was significantly higher than in other geographic regions studied to date.

We have discovered several interesting TUs, which deserve further attention. Three of them belong to the subfamily Leishmaniinae: TU***199*** is related to the genus *Novymonas*, while TU***196*** and TU***198*** are the most divergent and earliest branches within the subfamily. These TUs may represent new genera, however, in order to justify such classification, they would have to be available in culture, allowing their more thorough characterization (Votýpka et al., 2015). The other two remarkable trypanosomatids (TU***191*** and TU***197***) are associated with the genus *Strigomonas*, although the level of divergence of TU***197*** may be in favour of its distinct generic status. In any case, the overall scarcity of members of the highly interesting subfamily Strigomonadinae makes all new species worthy of detailed study.

The obtained data allowed us to estimate the diversity of parasites, prevalence of infection, host specificity and geographic distribution. The overall prevalence of infection in PNG (15%), was similar to that in China (16%), but significantly lower compared with 26–30% in Africa and the Neotropics (Maslov et al., 2010; Votýpka et al., 2010; Jirků et al., 2012). However, this difference may be explained by the different number of dissected bugs within various heteropteran families. In PNG, a significantly higher number of Miridae has been inspected for trypanosomatids. Since this group of very small true bugs is generally less frequently infected, the overall prevalence has been consequently reduced.

The new material also allowed delineation of specific and non-specific hosts for several widespread trypanosomatid species. However, it remains a mystery, which insects in PNG host *Leptomonas pyrrhocoris*, the best known cosmopolitan trypanosomatid.

As in previous reports (Votýpka et al., 2010, 2012a; Kozminsky et al., 2015), our study showed only partial association between the insect host families and trypanosomatids. Yet, results presented herein provide more support for host-parasite coevolution than the previous studies (with a caveat of a sampling bias).

This is to our knowledge, the first study on insect trypanosomatids east of Wallace's Line and the considerable predominance of novel TUs in our material demonstrates that our knowledge about the diversity of these flagellates is far from being comprehensive. It also represents first evidence that the extraordinary endemism of organisms inhabiting PNG is also inherent to parasitic protists. Moreover, our analysis has revealed several new clades within the tree of the Trypanosomatidae, representing putative new genera, which are worthy of further study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2019.09.004.

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